Vol. 148, No. 1 February, 1975

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

COPPER UPTAKE AND EXCRETION BY *BUSYCON CANALICULATUM* L.

Reference: *Biol. Bull.*, **148**: 1–15. (February, 1975)

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Copper concentration in tissues of the channeled whelk, *Busycon canaliculatum* L., despite great variability, has been found to undergo a seasonal cycle, generally increasing in the early summer, when feeding begins, and decreasing in the fall and in the winter hibernation period (Betzer and Pilson, 1974). This metal is physiologically important, since marine gastropods require copper for the synthesis of the blood pigment, hemocyanin (Hcy). Thus it is of interest to know how they accumulate the trace substance, to what degree its concentration in their bodies is regulated, and to what environmental factors the seasonal copper cycle may be related. In the present study, uptake of copper into the whelk, sites of accumulation, and the possibility of copper regulation have been investigated in a series of uptake experiments using radioactively labeled copper as a tracer. Measurements were made of possible copper loss by excretion and by spawning.

Materials and Methods

Fifty-four specimens of *Busycon canaliculatum* were obtained in Narragansett Bay, Rhode Island, and maintained in running bay water as described previously (Betzer and Pilson, 1974). Animal weights ranged from 100–200 g, in general.

Uptake experiments

Radioactive ^{64}Cu was prepared from copper shot dissolved in concentrated nitric acid and made to volume with deionized water to give a concentrated copper nitrate solution. Aliquots of this standard were neutron-activated, ordinarily for 6–8 hr, at a neutron flux of 4×10^{12} neutrons cm^{-2} sec^{-1} at the Rhode Island Nuclear Science Center.

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For experiments with ^{64}Cu uptake, a known amount of activated Cu standard was mixed into 3 liters of filtered (Whatman #42) freshly-collected, Narragansett Bay water in 4-liter polypropylene beakers. One whelk was placed in each beaker for the uptake period of 1–48 hr. At intervals during the uptake period, whelks were removed, put into square plastic refrigerator containers, and placed above a sodium-iodide crystal. Gamma emission from ^{64}Cu was counted, using a Tracerlab multichannel analyzer. All counts were corrected to time 0 using the decay constant for ^{64}Cu ($t_{\frac{1}{2}} = 12.8$ hours).

At the end of the uptake period, whole whelks were counted again and dissected. Whole organs (digestive gland, gut, kidney, gonad, osphradium, and gill), one-ml blood samples, and tissue samples (ordinarily foot muscle and mantle) were placed in preweighed plastic vials and counted in the well of the sodium-iodide crystal; the empty shells were also counted in refrigerator containers above the crystal. Each of the whole organs, blood and tissue samples was then weighed, dissolved, and analyzed for stable copper concentration by the spectrophotometric cuproine method or by atomic absorption spectroscopy, as described and reported previously (Betzer and Pilson, 1974).

An aliquot of activated copper standard equal to the amount added to each uptake beaker was counted in the crystal at the beginning of each experiment and the same aliquot was counted at the time of each subsequent count of whelks or tissues. The activity of the known amount of copper in the reference solution was used to convert all counts made in the well of the crystal to μg of labeled copper present in the substance counted.

A number of separations were carried out in trying to determine whether radioactive copper taken up in the blood had been incorporated in the hemocyanin molecule or was carried with a lower molecular weight fraction. The first technique used was ultracentrifugation (Ghiretti, 1966). A precounted labeled blood sample was diluted with 0.1 M KCl to fill the centrifuge tube and spun 1.5 hr at 360,000 g in a Beckman Ultracentrifuge. The content of radioisotope associated with the resulting pellet (containing the hemocyanin) and supernatant fluid was then determined. A second separation technique used was gel filtration. A 0.5 ml aliquot of each labeled precounted blood sample was applied to a column containing 30 ml of Sephadex® G-15 gel and eluted with 0.1 M KCl at a rate of 3.5–7 ml/hr. Fractions were collected automatically every 30 min and counted. The column was calibrated with a solution of unlabeled whelk blood and ^{64}Cu in seawater; fractions were collected, counted, and analyzed for protein by the Biuret method. The third technique for separating unbound copper from blood hemocyanin was that of Joselow and Dawson (1955), employing ion-exchange chromatography. Labeled precounted blood samples were applied to columns of Bio-Rex 40 (100–200 mesh) in the sodium form and passed through at about 5 ml/hr. The effluent was collected in 10-ml volumetric flasks and counted.

Excretion experiments

Sea water was passed through a Whatman #42 filter and then through a Chelex column to remove trace metals. Individual whelks were placed in polypropylene beakers containing 3 liters of this water and incubated up to 72 hr

as was a blank beaker, with no whelk added. After removal of the whelks, the copper content of the water and any particulate matter was determined; the walls of the beaker were rinsed with 200 ml of 2 N nitric acid and the copper content determined.

The copper content of the seawater used was determined by passing the water through a column containing Chelex-100 ion-exchange resin in the hydrogen form, at a rate of about 5 ml/min (Riley and Taylor, 1968). The column was rinsed with 50 ml of deionized water and eluted with 200 ml of 2 N nitric acid, which was boiled down to a few ml in a Vycor® beaker covered with a Teflon® watch glass. The acid rinses of the beaker walls and the particulate matter were similarly boiled down. The solutions were transferred to 25-ml volumetric flasks, made up to volume with deionized water, and analyzed for copper by atomic absorption spectroscopy. Nitric acid blanks were run and the values subtracted from the copper concentrations in the samples.

Results

Uptake of labeled dissolved copper by Busycon

In eight experiments a total of 34 whelks were exposed to concentrations of added copper of 3–6 μg/l (total copper concentration of 6–9 μg/l), comparable to the normal concentration of copper in Narragansett Bay water, 3 μg/l (D. Hallett, University of Rhode Island, personal communication). The amount of copper present in the incubation beakers was thus insignificant in comparison to the very large amount of total body copper in *Busycon* (averaging 7600 μg for a whelk of 100 grams—Betzer and Pilson, 1974). In one experiment, 4 whelks were exposed to high values of added copper, 106 μg/l. Incubation times for all the uptake experiments, between 1 and 48 hours, were limited by the short half-life of the isotope, 12.8 hours.

Sequence of uptake by the whole whelks. Because of the difference in geometry between whole whelks counted above the crystal and the 1 ml of reference solution counted in the well of the crystal, counts of whole whelks cannot be converted directly to μg of copper taken up by the animals. A conversion factor was obtained, however, using data from 4 experiments in which the activity of the whole uptake beakers, containing sea water and added labeled copper, was continued before and after incubation of the whelks. In each case a linear relationship was found between loss of activity in the beakers and gain in activity by the whelks which occupied them. Because the total amount of copper (μg) originally added to the beaker was known, the amount of copper taken up by a whelk could then be calculated from the slope of the graph. Since the activity (counts per minute) of the copper initially present in the beaker was proportional to the activity of the same amount of copper in the reference solution, a conversion factor was calculated from the counts per minute (cpm) in the whelk and the fraction of the copper in the reference solution represented by these counts. In four different uptake experiments the factor was calculated to be 4.20, 3.66, 4.26, and 3.79, giving an average of 4.0. This factor was applied in all uptake experiments to calculate, from counts of whole whelks above the crystal, the μg of labeled copper taken up

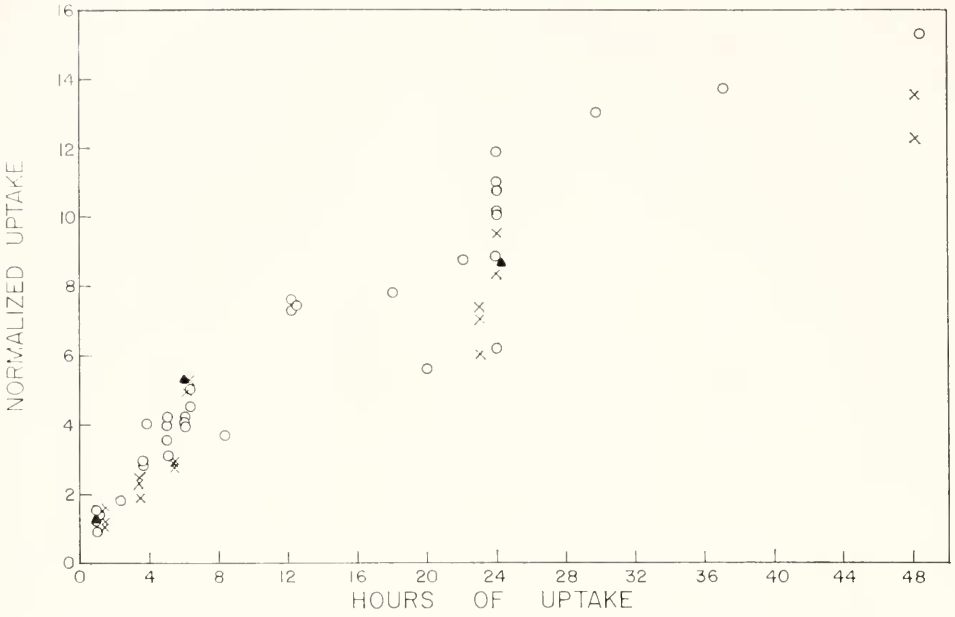

FIGURE 1. Uptake of dissolved, labeled Cu with time into whole whelks (weighing 100 g) from sea water. Points represent individual whelks exposed to different initial concentrations of added Cu in the beakers: diagonal crosses, 6 μg/l; open circles, 9 μg/l; closed triangles, 109 μg/l; temperature of experiments: 21° C.

by the whelks:

$$\frac{4.0\ (\text{cpm in whole whelk})\ (\text{total } \mu\text{g Cu in uptake beaker})}{\text{cpm of reference solution in crystal}} = \mu\text{g Cu taken up into whelk.}$$

The validity of the conversion depends on the active copper being distributed in and on the body with approximately the same geometry from one whelk to the next. Whelks were always placed in the same orientation to the crystal during counting. The conversion factor was also applied to whelk shells counted above the crystal after dissection.

To allow comparisons among experiments in which different copper concentrations were used in the uptake medium, the concentrations of labeled copper taken up in both whole whelks and tissues have been normalized by dividing by the initial concentration of copper in the medium. This is referred to as the normalized uptake:

$$\text{Normalized uptake} = \frac{\mu\text{g Cu taken up per g of whelk}}{\text{initial } \mu\text{g Cu per ml of water in beaker}}.$$

Figure 1 shows the typical sequence of copper uptake into whole whelks from seven uptake experiments. The whelks showed a smooth, continuous increase in

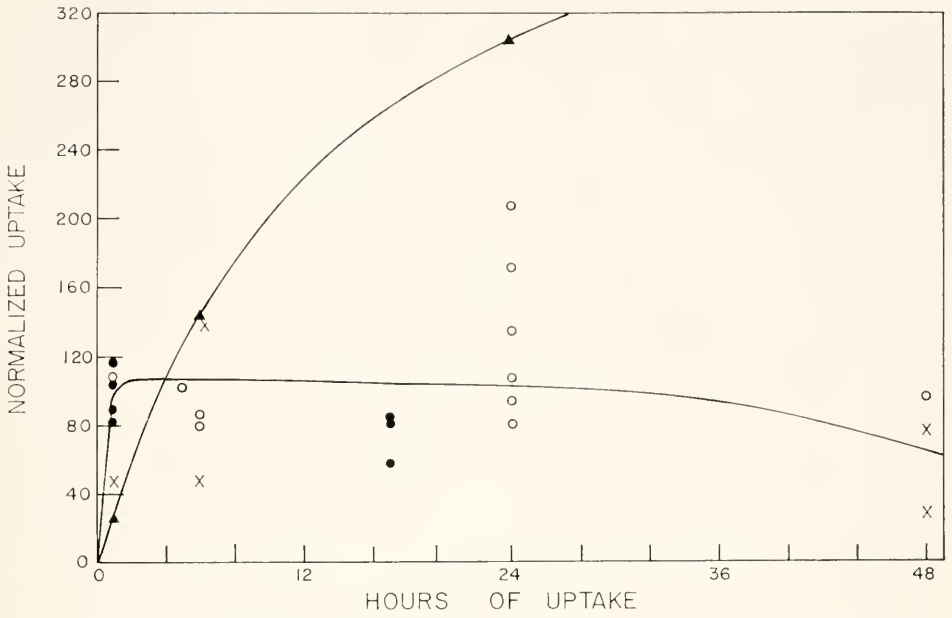

FIGURE 2. Uptake of dissolved, labeled Cu by gills with time. Points represent gills of individual whelks exposed to different initial Cu concentrations and temperatures of uptake: diagonal crosses, 6 μg/l and 17–21° C; open circles, 9 μg/l and 15–20° C; closed circles, 9 μg/l and 21–25° C; closed triangles, 109 μg/l and 15–20° C.

labeled copper, with the slope decreasing with time and leveling off toward 48 hr. This is probably due to a depletion of ^{64}Cu in the beaker; the three whelks incubated 48 hr had taken up 63%, 69%, and 78% of the total labeled copper originally present in the medium.

The effect of copper concentration on the rate of uptake into whole animals is apparent in Figure 1. Despite the individual variation, whelks exposed to 6, 9, and 109 μg Cu/l generally showed the same normalized uptake in the same length of time; *i.e.* uptake rate was directly proportional to the concentration of copper in the medium. Yager and Harry (1964) also found increased ^{64}Cu uptake in the freshwater snail, *Taphius glabratus,* when more copper was available.

Localization of copper taken up by whelks. Although the amount of labeled copper taken up into the individual tissues was small in comparison to their stable copper content, most tissues showed an accumulation of ^{64}Cu many times its initial concentration in the incubation medium. As in whole whelks, uptake rates in individual tissues and shells were directly proportional to copper concentration, except in the case of the gill and osphradium, as noted below.

Copper uptake onto the shell of *Busycon* followed the same general pattern as uptake by whole animals, with the normalized uptake increasing smoothly but with decreasing slope throughout the exposure period. This was not found by Yager and Harry (1964), whose measurements of ^{64}Cu uptake from 30 μg/l solutions by *Taphius glabratus* showed wide fluctuations and a decrease in copper

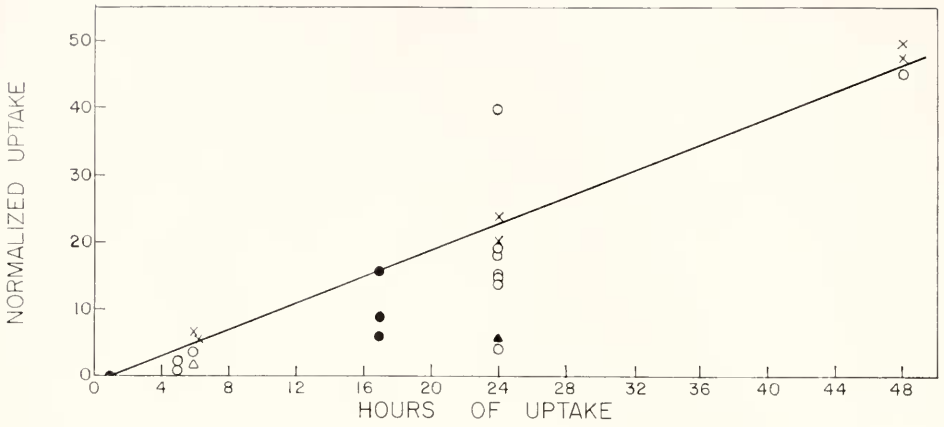

FIGURE 3. Uptake of labeled Cu by digestive gland with time. Points represent digestive glands of individual whelks exposed to different initial Cu concentrations and temperatures of uptake: diagonal crosses, 6 μg/l and 17–21° C; open circles, 9 μg/l and 15–20° C; closed circles, 9 μg/l and 21–25° C; closed triangles, 109 μg/l and 15–20° C. At 1 hr of uptake there are 6 points clustered at a normalized uptake of about 0.

concentration on the shell with time. In the present experiment the shell consistently accounted for about 30% of the total counts of the whole animal, and this copper was adsorbed on the surface. Counts of shells with a portable counter, after the whelk tissues had been dissected out, showed essentially all the activity on the outside. An empty shell incubated as a control in one experiment showed the same uptake rate as the shells of live whelks, suggesting that the inner nacreous layer adsorbed little copper in comparison to the hairy, organic periostracum.

Of the soft tissues, the gills (Fig. 2) and osphradium were the first to become strongly labeled. At low levels of copper in the medium, comparable to those of the environment, the gills had a normalized uptake of about 100 after 1 hr of incubation and may already have been saturated with copper. After this time the concentration in the gill showed little change, except for a possible decrease between 24 and 48 hr when the supply of labeled copper in the medium had become depleted. No difference in normalized uptake was distinguishable between the gills of whelks at 6 and 9 μg Cu/l; but gills of whelks at 109 μg/l showed a different pattern: not yet saturated at 1 hr, they continued to accumulate copper up to 24 hr, reaching the much higher normalized uptake of 300. The osphradium, a very small (0.1–0.2 g), gill-like structure adjacent to the gill in the mantle cavity, differed from the gill in showing a more gradual accumulation of copper with time. It also reached a much higher normalized uptake, leveling off at about 400 by 24 hr.

Despite individual variation, the kidney showed a fairly steady linear increase in labeled copper with time. The first of the internal organs to show significant uptake, it exhibited a concentration greater than or equal to that of the medium after 1 hr of incubation; by 24 hr it had a normalized uptake of 15–20. The gut and digestive gland (Fig. 3) showed little evidence of copper absorption at 1 hr;

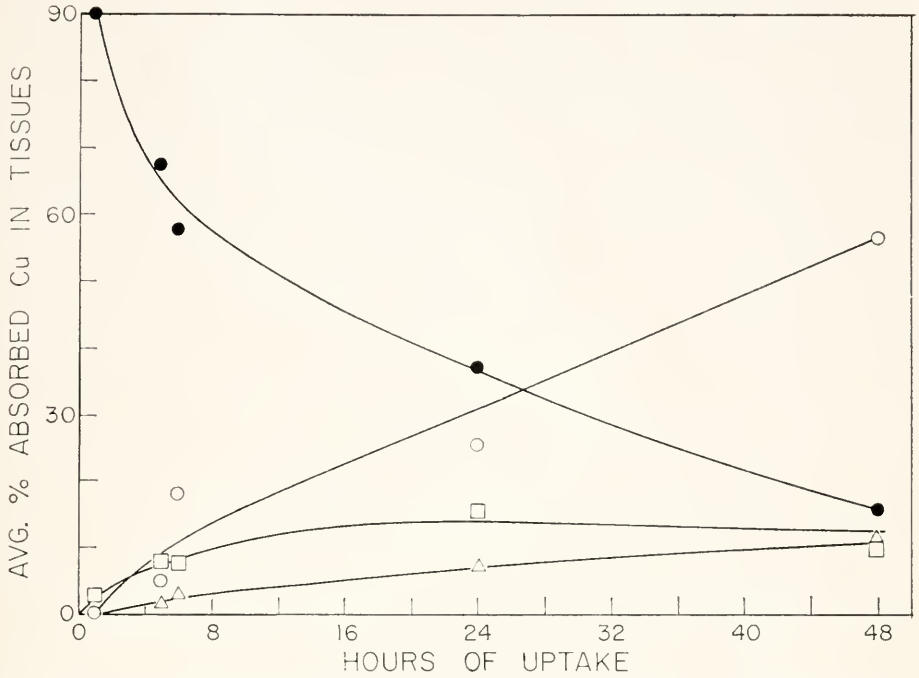

FIGURE 4. Sequence of uptake of Cu with time into the gill and tissues of the visceral mass of 18 whelks exposed to 6–9 μg Cu/l at a temperature of 15–20° C. The total Cu taken up into the gill, osphradium, digestive gland, gut, kidney, and gonad was summed, and the percentage contained in each tissue was calculated for each whelk. The average percentages for each uptake period were then plotted against the duration of uptake. Only the most quantitatively important tissues are shown: closed circles, average percentage in gill; squares, average percentage in kidney; open circles, average percentage in digestive gland; open triangles, average percentage in gut.

what little activity was found may have been due to the presence of blood. By 6 hr the normalized uptake was about 5 in both, and uptake continued at an approximately linear rate in both tissues for the rest of the incubation period, reaching a similar normalized uptake of about 45–60 by 48 hr, with no sign of leveling off.

The blood was labeled during the first hour, with a concentration of absorbed ^{64}Cu about equal to that initially present in the medium. More ^{64}Cu accumulated with time, but, in general, ^{64}Cu concentration in the blood after any exposure time (after 1 hr) was lower than in the kidney, digestive gland, or gut. Centrifuging blood samples to remove the cells was found not to affect the ^{64}Cu activity in the sample, indicating that the blood cells were not significant sites of copper accumulation.

Other tissues were routinely counted for copper uptake: foot muscle showed a low level of label, probably due mostly to the presence of blood; tissue exposed directly to sea water, such as the mantle and mucous gland, showed a slow increase in activity, probably due to surface adsorption as well as blood; the gonad was extremely variable but showed generally about the same concentration of active copper as the digestive gland and gut.

The metabolically active gill, osphradium, and visceral mass tissues together contained an average of 62% of the ^{64}Cu in *Busycon* tissues after 1 hour of incubation; they contained an average of 45% (range 30–50%) after 5–48 hours of incubation (as copper was accumulated in the blood and foot muscle). The sequence of changing distribution of absorbed copper among these metabolically active tissues is shown in Figure 4. Here, the total copper taken up into the gill, osphradium, digestive gland, gut, kidney, and gonad was summed, and the percentage found in the various tissues averaged for the different uptake periods. The total in the blood could not be included because the blood volume was not known. Because the speed of uptake in some tissues appeared to vary with temperature and copper concentration in the medium, data are presented here only for whelks exposed to 6–9 μg Cu/l, at temperatures of 15–20° C. At 1 hr of exposure, the gill had 90% of the total copper in these tissues, but although its total copper remained more or less constant (Fig. 2) it decreased in importance as copper in the other tissues rose, so that at 48 hr, the gill represented only about 15% of the total. The digestive gland had taken up negligible copper by 1 hr, but showed a steady increase in importance, so that it contained 50% of the copper at 48 hr. The kidney appeared to increase until about 24 hr, after which it leveled off or decreased slightly. The gut showed a slow increase over the 48-hr period, containing at the end of this time about 10% of the copper absorbed in these tissues. This pattern suggests that copper moves into the body at the gills and is transferred mainly to the digestive gland, although it is distributed to other tissues of the visceral mass as well.

This explanation is supported by the results of an "uptake and release" experiment. Three whelks which had been exposed to a concentration of 6 μg/l added labeled copper for 17 hr were counted whole and then transferred to Bay water with 6 μg/l added unlabeled copper for 24 hr before dissection and counting. Counts of radioactive copper in these whelks kept in unlabeled water for 24 hr after uptake were as high as at the end of the uptake period, showing that absorbed copper was not rapidly flushed out. Analysis of active copper in the water and particulate matter in the "release" beakers, after the removal of the whelks, showed that in all three whelks only about 7% of the label that had been taken up was released (an amount that was within the counting error for whole whelks). The absorbed copper was redistributed among the tissues during the 24 hr incubation in unlabeled water, in comparison with that of three other whelks dissected immediately after the 17 hr of uptake. Labeled copper in the gills decreased to less than half its concentration at 17 hr, while it increased strikingly in the kidney, gut, digestive gland, and gonad. There was no change in labeled copper levels in the blood. In terms of percentage of total labeled copper in gills, osphradium, and visceral mass tissue, the digestive gland increased from 15% to 51%.

Form of labeled copper in the blood and digestive gland. Ultracentrifugation of blood samples from 11 whelks from the uptake experiments yielded pellets (Hcy fraction) containing an average of 2/3 of the total label in the blood. Even when an excess of neutral, unlabeled copper was added to the sample before centrifugation, the bulk of the activity came down with the Hcy (and thus was not displaced). When labeled ionic copper was added to a sample of fresh whelk blood, 43% of the activity came down with the Hcy. This showed that the Hcy could bind or adsorb excess copper.

TABLE I.

Cu excretion by individual whelks

Excretion into water from which Cu had been removed

	Length of incubation	Total Cu released, μg	Cu excreted per 24 hr per 100 g
A. Feb. 1971	24 hr	7.83	10.44
T = 25° C	48 hr	7.41	4.07
	72 hr	9.73	4.63
	72 hr	19.02	9.91
B. April 1971	$6\frac{1}{4}$ hr	4.00	18.6
T = 17–19° C	24 hr	3.16	2.75
	48 hr	9.60	4.00
C. July 1971	6 hr	3.67	12.5
T = 21° C	24 hr	3.24	2.42
Aeration provided	48 hr	8.54	2.81
			$\bar{X}$ = 7.21 μg Cu/100 g per day

Excretion and uptake in water with added labeled Cu

	μg labeled Cu added	Length of incubation	Total Cu, μg, in beaker	Total minus blank	μg labeled Cu taken up by whelk*	Total Cu excreted	Cu excreted per 24 hr per 100 g
D. April 1971	17.6 μg	6 hr	17.8	−0.9	3.6	2.7	13.3
		24 hr	19.5	+0.7**	9.3	10.0	9.61
		48 hr	14.7	−4.0	12.1	8.1	3.51
		48 hr (no whelk) (blank)	18.7	—	—	—	—
E. July 1971	18 μg	6 hr	14.61	−4.85	9.06	4.21	6.86
		24 hr	17.70	−1.76	12.79	11.03	6.18
		48 hr	18.96	−0.50	14.09	13.59	5.96
		48 hr (no whelk) (blank)	19.46	—	—	—	—
							$\bar{X}$ = 7.57 μg Cu/100 g per day

* results from counts of radioactivity in uptake experiment.
** net excretion; all others show net uptake.

Labeled blood samples from 6 other whelks were passed through columns of Sephadex® gel; in each case the radioactive copper came through in the fraction containing the Hcy. Dissolved ionic copper and Hcy applied separately to Sephadex columns came through in different volume fractions, because of their difference in molecular weight; but when ^{64}Cu in sea water was mixed with a solution of unlabeled whelk blood, the copper activity came through in the same fraction with the Hcy. Thus the Sephadex gel filtration technique cannot distinguish the excess

copper nonselectively bound by Hcy from copper incorporated in the active site of the Hcy molecule.

A similar ability to bind excess copper was found for lobster Hcy by Johnston and Barber (1969). They reconstituted lobster apohemocyanin with copper sulfate and with hepatopancreas supernatant and found that in the presence of excess copper, the apohemocyanin bound essentially all available copper—up to 6 times its original copper content.

In calibration experiments with BioRex 40 ion-exchange resin, *Busycon* blood passed through the column with its copper content unchanged, and labeled copper applied to the column in aqueous solution was quantitatively (99.8%) retained on the column. When labeled copper was mixed with *Busycon* blood, only a small amount of labeled copper (probably too small to have been detected with the counting techniques of Joselow and Dawson, 1955) passed through the column, 0.1–0.2 μg of a total of 3–10 μg copper applied.

In BioRex column separations of labeled blood samples from 11 whelks from the uptake experiments, a very small amount of labeled copper came through with the Hcy, 0–0.02 μg, or 0–37% of the initial labeled copper in the samples. There was no apparent correlation between the length of the uptake period and the amount of label that came through. Because this was less than 10% of the copper which had leaked through the columns in the calibration experiments, it is not known whether it represents incorporation in the Hcy, or "leakage" by the column. Since even with these small quantities of labeled copper, 63–100% of the labeled copper was removed from the blood sample by the column, it seems most likely that the labeled copper found in the blood in the uptake experiments was nonspecifically bound to the Hcy, not incorporated in the Hcy molecule.

A preliminary determination of subcellular distribution of copper in the digestive gland was made for two whelks incubated 24 hr with 6 μg/l added labeled copper. Differential centrifugation of the homogenized gland was followed by counts of each fraction for ^{64}Cu and analysis of each fraction for total copper. It was found that the newly taken up labeled copper was distributed differently than the total stable copper; 32–37% of the labeled copper remained in the supernatant after ultracentrifugation at 100,000 $\times$ g for 1 hour, while only 5–10% of the stable copper remained in this fraction, even the hemocyanin having sedimented. This indicated that a significant fraction of the newly absorbed copper was bound in some way to lower molecular weight substances, preventing the nonspecific binding that occurred between ionic copper and hemocyanin in previous centrifugation experiments and that probably occurs in transport of uptaken ionic copper by the blood.

Copper excretion by Busycon

A series of excretion or release experiments was carried out in order to determine whether the uptake of labeled copper by *Busycon* represented a net accumulation of copper or an exchange between the labeled copper in solution and the much larger pool of unlabeled copper in the whelk. Table 1 (A-C) presents the results of three experiments in which whelks were incubated in water previously stripped of trace metals. The net daily excretion per 100 g of soft tissue weight

ranged from 2.42–18.6 μg copper, with a mean of 7.21 ± 5.42 (1 σ). This value is not corrected for possible uptake by the whelks of copper they released; if this occurred, the total excretion would be greater. This could be the explanation for the higher rate of excretion by the whelks incubated for 6 hr in both Exps. B and C; by 6 hr only a comparatively small amount of copper had been released, and since rate of uptake is proportional to concentration, the competing effect of uptake on excretion was not seen until later.

Excretion was also measured in two of the uptake experiments described in the preceding section. After the removal of trace metals from the incubation medium, low concentrations of labeled copper were added; this made possible the simultaneous measurement of uptake and excretion. After the incubation period, the total copper in the water and particulate matter and on the walls of the beakers was determined and combined with the uptake data to give an estimate of total excretion. It has been assumed that copper taken up was not excreted, which seems reasonable, from the small amount of labeled copper lost in the uptake and release experiment. The results are presented in Table I (D, E). Daily copper excretion per 100 g of whelk ranged from 3.51–13.3 μg, averaging 7.57 ± 3.42 μg. This is roughly equivalent to the daily copper excretion when no copper was added and suggests that addition of copper to the medium did not increase the excretion rate. Since the copper excretion rate was about the same whether or not there was any initial copper in the water it seems likely that the copper which appeared was really excreted and not just desorbed from the shell surface. In the experiments where copper was added to the water the whelks maintained or increased their total body copper, instead of losing copper to the medium. Since the concentration of added copper in these experiments (6 μg/l) was not too different from that in the bay (3 μg/l), these results indicate that under ordinary conditions whelks probably remain more or less in balance, with dissolved copper taken in being equaled or only slightly exceeded by copper excreted. Where environmental copper concentrations are high, however, whelks may be expected to show a net accumulation from the medium, in addition to whatever copper is supplied in the diet.

Narcotizing with the gastropod relaxant and insecticide, Sevin®, according to the procedure of Carriker (1963), was found to have a pronounced effect on copper excretion by *Busycon*. In two uptake experiments, a whelk was narcotized with Sevin® before and during exposure to labeled copper. The whelks were dissected at 24 and 48 hr. Both whelks took up significantly less label than the other animals, and the shell of the 48-hr whelk had reduced label compared to the shell of normal whelks. In both narcotized whelks, uptake of labeled copper into internal tissues was negligible, and even in gills and osphradium, uptake was significantly reduced. These data were explained when the copper content of the beakers used for the 24 hr experiment was determined. The average of the other 5 beakers was 47 μg; the beaker which contained the narcotized whelk had 650 μg of copper. The Sevin® stock solution was analyzed; the 3.5 ml used in this experiment would have added only 0.245 μg copper to the beaker. The 650 μg of copper thus represented an excretion rate of 554 μg/day per 100 g tissue, about 75 times greater than the rates recorded in the excretion experiments. This massive release of unlabeled copper would tend to displace adsorbed labeled copper on the

shell and body. The kidneys of the 2 narcotized whelks showed unusually low stable copper concentrations: that of the 48 hr whelk had 13 μg/g, and that of the 24 hr whelk had 20 μg/g, 40% and 30%, respectively, of the average concentrations in the kidneys of the other four whelks in each experiment, reported (as Feb. and Mar. averages) by Betzer and Pilson (1974).

Loss of copper through spawning. It was thought that the autumn reduction of the copper concentration in the blood and tissues of *Busycon* could be due to the fall spawning. In *Busycon,* eggs are fertilized internally and shed by the female enclosed in capsules connected as a long chain or egg string. Development from the egg to the juvenile stage takes place within the capsule. A measure of possible copper loss through spawning was made by determining the amount of copper in the contents of individual capsules obtained in late October, when the larvae had developed to the veliger stage. The larvae and associated albuminous material from ten capsules were ashed, dissolved in concentrated HCl, rinsed into volumetric flasks, and the copper content determined by the cuproine method (Diehl and Smith, 1958). The number of larvae per capsule ranged from 65–96, averaging 83. The copper present in the capsule contents ranged from 10–45 μg, averaging 23 μg. Thus, assuming 100 capsules/egg string (a reasonable average, from the data of Magalhaes, 1948), and no copper in the walls of the capsules (which were not analyzed), 2300 μg would be shed at spawning. This copper loss is much greater than the amount of change between pre- and post-spawning reproductive organs—less than 100 μg in a 120-g whelk (Betzer and Pilson, 1974), suggesting that perhaps copper from other tissues is mobilized and deposited in the eggs before spawning. Whether a similar loss of copper might occur in the male is not known.

Discussion

The fate of ^{64}Cu absorbed in *Busycon* agrees fairly well with that shown by studies of heavy metal uptake by a few other organisms (Bryan, 1964, 1968; Bryan and Ward, 1965; Hobden, 1969). In 6–42 hr of uptake of ^{64}Cu by the freshwater snail *Taphius glabratus* from solutions with a concentration of 31 μg Cu/l, Yager and Harry (1964) found the same magnitude of concentration in all tissues, except that in the liver it was 4–7 times higher. Townsley (1964) found that ^{64}Cu injected into the body of *Busycon* accumulated in the digestive gland.

From the pattern of uptake with time and the results of the "uptake and release" experiment, it seems that in *Busycon* the gills are probably the primary site of copper absorption from sea water, as they have been shown to be for other metals in other organisms (Bryan, 1968; Bryan and Ward, 1965). In whelks exposed to low, environmental concentrations of dissolved copper, the gills reached an "equilibrium" concentration by one hour, suggesting a balance between the rate of uptake by the gills and the rate of transport away to the other tissues. The higher normalized concentrations reached in the gills exposed to high copper concentration (109 μg/l) could be evidence that the mechanism of transport of copper from the gills into the body has become saturated at this concentration.

As discussed by Bryan (1968), it is not necessary to postulate active uptake of zinc by the gill of the lobster, *Homarus,* since most of the zinc in the blood and tissues is bound to a protein fraction; thus there is a concentration gradient of

unbound zinc—higher in the medium, and lower in the gill. A similar argument may hold for *Busycon*; the results of the blood centrifugation, gel filtration, and column separations show that ionic copper is strongly bound by the blood Hcy of whelks, so that the concentration of ionic ^{64}Cu in the gill may actually be much lower than in the medium, regardless of the high concentration of total labeled copper.

The transfer of much of the absorbed copper to the digestive gland may represent a way of buffering the blood against high copper concentrations. Bryan (1964), in experiments with zinc uptake in *Homarus*, described the hepatopancreas as a "sponge which mops up excess zinc from the blood, and so, with the excretory organs, helps to keep the blood Zn level fairly normal" (page 556). We do not know whether the process of copper absorption by the digestive gland of *Busycon* is a regulated one, or a nonspecific uptake of the excess copper. The preliminary experiments with subcellular fractionation of the labeled digestive gland indicate that a substantial fraction of the copper newly taken up is held in association with low molecular weight substances—a different form than that in which it seems to be carried in the blood.

While the marked increase of copper in *Busycon* tissues during the early summer can be accounted for by the commencement of feeding (Betzer and Pilson, 1974), the mechanism for the drop in the fall and winter is not so easily explained. Typical copper loss from the blood (estimating blood volume as 30% as in *Buccinum*—Staaland, 1970) and digestive gland of an average whelk with tissue weight of 120 g can be calculated from the data of Betzer (1972) to yield a decrease of about 11,000 μg between summer and winter. Spawning was calculated to release perhaps about 2300 μg of copper. The rate of excretion, about 7 μg/day for a 100 g whelk, was balanced by the rate of uptake of dissolved copper in the incubation experiments, so that if excretion occurs at the same rate in the fall as in these experiments (conducted in the spring and summer), a large amount of copper could not be lost by this route. The striking rise in kidney copper concentration found by Betzer and Pilson (1974) in the late summer and early fall, when the other tissues are decreasing, however, indicates that perhaps increased copper excretion is occurring at this time of year. The copper content of three kidneys noticed for their unusual dark blue color at this season was more than five times higher than in typical winter animals. This could be evidence of a massive release of copper from apparently healthy fall whelks.

It appears that, in general, the copper content of *Busycon* is only very crudely regulated. Highly variable amounts of copper are undoubtedly consumed in the whelk diet, depending on the proportion of fish, shellfish, or other invertebrates consumed. Dissolved copper is taken up at apparently unregulated rates, depending on the concentration of the medium, so that it could be accumulated from polluted environments. Excretion of copper appears to be generally low and not related to the copper concentrations in the medium, although massive release of copper via the kidney in the fall could represent a coarse control on body copper concentration, along with release of copper at spawning. The highly variable tissue copper concentrations found in *Busycon* at all seasons of the year (Betzer and Pilson, 1974) attest to the small degree of regulation of this highly concentrated and (theoretically) metabolically important trace metal.

We wish to thank Richard Sisson, of the R. I. Dept. of Natural Resources, and Stanley Spink for providing specimens of *Busycon*. Stanley Cobb, Ross Wilcox, Kent Fanning, Patricia Kremer, and Jim Kremer helped in tending and feeding whelks and recording observations during long-term experiments. Robert Duce kindly made available his Perkin-Elmer 303 atomic absorption spectrophotometer; and George Tremblay, the Beckman Ultracentrifuge and other facilities of the Biochemistry Department of the University of Rhode Island. We are indebted to Stuart Kupferman for the suggestion of using radioactive labeling in tracing copper uptake, and to Donald Phelps, of the Environmental Protection Agency, and the staff of the R. I. Nuclear Science Center, particularly Mike Doyle and Frank DiMeglio, for use of the nuclear reactor and its laboratory facilities and for technical assistance. Peter Betzer assisted in many of the experiments, performed numerous copper analyses, and gave helpful suggestions throughout the preparation of the manuscript. This work was performed at the Graduate School of Oceanography of the University of Rhode Island, while one of us (S.B.B.) was supported by a National Science Foundation Graduate Fellowship.

Summary

1. Pathways of copper into *Busycon canaliculatum* and sites of accumulation were investigated in uptake experiments using ^{64}Cu. Routes of possible copper loss were investigated in excretion experiments and by determination of copper content of egg capsules.

2. Uptake of dissolved ^{64}Cu by 38 whelks followed a smooth curve, slowing with time; about 2/3 of the available ^{64}Cu in 3 l of water was absorbed by 48 hr. The rate of uptake was proportional to the concentration of the medium.

3. Among the soft tissues, ^{64}Cu appeared first on the gills, which in 1 hr reached a normalized concentration 100 times that initially present in the medium, and in the blood and kidney (normalized concentration = 1 at 1 hr). By 6 hr of exposure, ^{64}Cu appeared in the gut and digestive gland (normalized concentration = 5).

4. The ^{64}Cu continued to accumulate in the digestive gland, so that by 48 hr, this tissue contained 50% of the total copper taken up by the gill and organs of the visceral mass. Transfer of absorbed copper to the digestive gland continued even when whelks were removed to unlabeled sea water for 24 hr.

5. Separations carried out on blood from whelks labeled with ^{64}Cu indicated that the absorbed copper in the blood was nonspecifically bound to hemocyanin.

6. Excretion rates for copper averaged 7 μg/24 hr per 100 g fresh tissue weight, and appeared unaffected by the copper concentration of the medium. Under normal environmental copper concentrations, rates of dissolved copper uptake and of copper excretion are probably about equal.

7. The average copper content of egg capsules was 23 μg/capsule. Spawning may be a significant route for copper loss, and an increase in copper excretion in autumn is also suggested as an explanation for a drop in tissue copper concentrations at this season.

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